

an important phenomenon in butterflies (Willmott & Freitas, 2006; Janz *et al.*, 2006). In Nymphalinae, recent studies have shown that the plasticity in host plants ranges may be related to diversification processes (Weingartner *et al.*, 2006; Nylin & Wahlberg, 2008). In this sense, *H. bella* may be a model for understanding the importance of local adaptation in the evolution of host plant use in Neotropical butterflies.

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LITERATURE CITED

- ALAYO, P. & L. HERNÁNDEZ. 1987. Atlas de las mariposas diurnas de Cuba (Lepidoptera: Rhopalocera). Editorial Científico-Técnica, La Habana.
- BECCALONI, G. W., S. K. HALL, A. L. VILORIA & G. S. ROBINSON. 2008. Catalogue of the hostplants of the Neotropical butterflies / Catálogo de las plantas huésped de las mariposas Neotropicales. In: m3m - Monografías Tercer Milenio, Vol. 8. S.E.A., RIBES-CYTED, The Natural History Museum, Instituto Venezolano de Investigaciones Científicas, Zaragoza.
- BIEZANKO, C. M. 1949. Acraeidae, Heliconiidae et Nymphalidae de Pelotas e seus arredores (Contribuição ao conhecimento da fisiografia do Rio Grande do Sul). Livraria do Globo, Pelotas.
- BROWN, K. S., JR. 1992. Borboletas da Serra do Japi: Diversidade, habitats, recursos alimentares e variação temporal, p. 142-187. In: L. P. C. MORELLATO (ed.). História Natural da Serra do Japi: Ecologia e preservação de uma área florestal no sudeste do Brasil. UNICAMP/FAPESP, Campinas.
- D'ABRERA B. L. 1987. Butterflies of the Neotropical Region. Part IV. Nymphalidae (partim). Black Rock Hill House, Victoria.
- DEVRIES, P. J. 1987. The butterflies of Costa Rica and their Natural History. Princeton University Press, New Jersey.
- HAYWARD, K. J. 1969. Datos para el estudio de la ontogenia de lepidópteros argentinos. Miscelánea. Instituto Miguel Lillo. Universidad Nacional de Tucumán 31:1-142.
- JANZ, N., S. NYLIN & N. WAHLBERG. 2006. Diversity begets diversity: host expansions and the diversification of plant-feeding insects. BMC Evolutionary Biology 6: 4.
- LORENZI, H. 2000. Plantas daninhas do Brasil: terrestre, aquáticas, parasitas, tóxicas e medicinais. 3rd ed. Editora Plantarum, Nova Odessa.
- NYLIN, S. & N. WAHLBERG. 2008. Does plasticity drive speciation? Host plant shifts and diversification in nymphaline butterflies (Lepidoptera: Nymphalidae) during the tertiary. Biological Journal of the Linnean Society 94: 115-130.
- SCOTT, J. A. 1986. The Butterflies of North America: a natural history and field guide. Stanford University Press, Stanford.
- TOLEDO, Z. D. A. 1973. Fauna del noroeste argentino. Contribución al conocimiento de los lepidópteros argentinos III. *Hypanartia bella* (F.) (Rhopalocera-Nymphalidae). Acta Zoológica Lilloana 30: 23-35.
- TOLMAN, T., & R. LEWINGTON. 1997. Butterflies of Britain and Europe. Harper Collins, London.
- WEINGARTER, E., N. WAHLBERG & S. NYLIN. 2006. Dynamics of host plant use and species diversity in *Polygonia* butterflies (Nymphalidae). Journal of Evolutionary Biology 19: 483-491.
- WILLMOTT, K. R., J. P. W. HALL & G. LAMAS. 2001. Systematics of *Hypanartia* (Lepidoptera: Nymphalidae: Nymphalinae), with a test for geographical speciation mechanisms in the Andes. Systematic Entomology 26: 369-399.
- WILLMOTT, K. R. & A. V. L. FREITAS. 2006. Higher-level phylogeny of the Ithomiinae (Lepidoptera: Nymphalidae): classification, patterns of larval hostplant colonization and diversification. Cladistics 22: 297-368.
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Duration of molt in a Neotropical butterfly (Lepidoptera: Nymphalidae)

In this note I present the results of an observational study on the length of molting in caterpillars of *Hypothyris ninonia daeta* (Boisduval, 1836), an ithomiine (Lepidoptera: Nymphalidae: Ithomiinae) butterfly of southeastern Brazil. To determine the proportion of time spent molting I measured the time caterpillars spend in molting relative to total development time. I was able to do this using a specific external marker (see below) that enabled me to recognize the start and the end of each molt period.

The species, hereafter referred to as *Hypothyris*, is a

medium-sized (forewing length 29-32 mm), relatively common butterfly inhabiting the Reserva Biológica do Poço D'Anta (RPA), a 277 ha forest fragment in Juiz de Fora, MG, Brazil (21°45'S, 43°20'W) (Costa, 2002).

Hypothyris has five larval instars that feed exclusively on *Solanum* (Solanaceae) species (Costa, 1999). Ovipositing females lay their eggs singly on the underside of hostplant leaves. Neonates are approximately 1.5-2 mm in length and are translucent white. Their color turns from white to greenish after feeding. However, since food consumption

stops during the molt process, and the larva partially evacuated its gut in preparation for molt, the distal end of the body changes coloration from a characteristic gray-green to translucent white. This color change of the last larval body segments thus provides a simple and reliable external marker that indicates the start of each larval molt, thereby facilitating the precise determination of the duration of each molt.

The color change mediated by gut contents does not occur in caterpillar species that have a pigmented or otherwise opaque body wall. For such cases, it is difficult or impossible to determine the start of molt process based upon visual inspection. Consequently, *Hypothyris* larvae provide an ideal subject for the study of molting behavior. For the pupal molt, color changes from gray-green to brownish and then to whitish. In all cases, shedding of the old head capsule marks the end of molt.

I collected *Hypothyris* immatures from a previously used study site at RPA (Costa, 1996) with all eggs and larvae between 21.IV (n = 14) and 1.V.1995 (n = 2). The 16 specimens were reared under semi-natural conditions, indoors, subjected to natural photoperiod and air temperature fluctuations, between 22.IV and 23.VI.1995 through adult emergence (8 ♀♀, 5 ♂♂). Each larva was reared individually in a glass jar with moist filter paper. Larvae were fed *Solanum cernuum* Vell., an abundant hostplant (Costa, 1999). Frass was removed with leaves and moisture were supplied as necessary.

The data for the study were from: (1) five immatures individuals (4 ♀♀: # B7, B8, B10, B12; 1 ♂: B5) reared from egg to adulthood (used to compute species-specific parameters); (2) four immatures individuals (B1, B2, B3, B4) that were collected as larva (three L3, one L5) and reared to adulthood; (3) two immatures individuals (B9, B11) reared from egg to adulthood but that molted to an extra sixth instar before pupation; and (4) two immatures individuals (P1, P2) collected as eggs on 1.V and that were reared to adulthood in different (plastic) containers. The other three immatures (B6, B13, B14) collected as eggs on 21.IV died during their second instar.

All caterpillars were examined by means of a magnifying lens 3-6 times daily for approximately 1-3 minutes each to determine the time spent during each larval instar during both growth (feeding) and molting (non-feeding) periods. This resulted in 139 surveys between 22.IV and 29.V.1995, when the last larva pupated. For each determination, I categorized caterpillar behavior as either "growth" (feeding, resting, moving around) or molting. I also noted activities such as mode and place of feeding, mode and place of resting, body aspect during molting,

etc. For computation, I assumed that all changes between growth and molting behavior occurred at the midpoint of the time interval between any two consecutive surveys. Larval size (body length to the nearest 0.5 mm) was measured at least once during each instar.

The climate at Juiz de Fora is characterized by a warm rainy summer and a cool dry winter. The average monthly temperature is 18.7°C, varying from 21.6°C (February) to 16.1°C (July). The diurnal range in air temperature greatly exceeded this average monthly change of only 5.5°C. On the other side, the monthly distribution of precipitation is very unequal throughout the year, varying from 272 mm (January) to 19 mm (July). Mean annual precipitation is ~1,500 mm (Costa, 1991).

To test whether the duration of the molt period is a function of the growth period of the same instar, I used linear regression analysis (least square). As a complement, I also used regression analysis to test if the time spent in each stage of development is a constant proportion of the total developmental time. Statistics (tests, symbols, and terminology) followed Sokal and Rohlf (1981)

Eleven of thirteen reared larvae had the usual five larval stages, but two caterpillars molted to an extra sixth instar before pupation. The latter two larvae were much smaller in body length (ca. 15 mm) when they underwent the extra instar. These exceptions are concordant with the hypothesis that there is a critical minimal length for a caterpillar prior to pupation (Nijhout, 1975; see also Esperk *et al.*, 2007).

Table 1 gives the average time in the growth stage of *Hypothyris* caterpillars increasing with successive

Table 1. The time duration of *Hypothyris* caterpillars during different instars (L1-L5) and corresponding molts. Values are means \pm SD from five larvae.

Phase (length, mm)	Duration \pm SD (hours)
L1 (1.5-3.5)	73.31 \pm 6.85
L1-L2 molt	14.45 \pm 5.21
L2 (3.5-5.5)	118.75 \pm 54.69
L2-L3 molt	21.14 \pm 2.55
L3 (6.0-8.0)	119.11 \pm 50.72
L3-L4 molt	20.65 \pm 3.26
L4 (8.5-13.0)	116.51 \pm 23.07
L4-L5 molt	28.03 \pm 3.25
L5 (13.5-27.0)	169.84 \pm 23.33
L5-pupa molt	36.93 \pm 6.41

larval instars, changing from 73.31 hours \pm 6.85 SD in L1 to 169.84 hours \pm 23.33 SD in L5. In parallel, the average time spent in the molt stage also increased with larval instar, changing from 14.45 hours \pm 5.21 SD (L1-L2 molt) to 36.93 hours \pm 6.41 SD (L5-pupa molt, including prepupa). However, considering larvae B9 and B11, these values are higher: 212.53 hours \pm 43.85 SD (L6) and 41.4 hours \pm 11.58 SD (L6-pupal molt).

The duration of growth and molting periods of the same instar are related; longer feeding periods correspond to longer molting periods (linear regression analysis for data of Table 1: $R^2 = 0.8544$; $t = 4.19$; $p < 0.05$). This relationship remains significant even including in the regression analysis data from all 13 caterpillars (Fig. 1).

The results of the regression analysis with data of Table 1 indicate that approximately 85% of the variation in the length of molting was explained by variation by duration of growth. The result appears relevant and more so considering that the sample included caterpillars from different instars growing simultaneously under a naturally fluctuating daily temperature regime.

In relative terms, the duration of molt periods varied from 14.7% (L3-L4 molt) to 19.4% (L4-L5 molt) of the total time that larva spent in each instar period (Table 1). However, the percentage of time spent by caterpillars during molting was not a function of the total duration (growth plus molting) of each instar (linear regression analysis: $R^2 = 0.098$; $t = 0.57$; $p > 0.9$), as would be expected if molt duration were

a fixed proportion of instar length.

Like other ectotherms (e.g., Casey, 1993), the performance of *Hypothyris* caterpillars is temperature-sensitive and varies in different months of the year. For instance, studies on the performance of these caterpillars in the field have shown that the growth rate increases linearly with temperature (unpublished data). Thus, the results of this study, which were obtained during autumn when the number of immatures in the field is decreasing, may overestimate the values during warm seasons, spring and especially summer, when immature density is higher (Costa, 1991) and development is probably faster. I believe the results of this study show that molting and growing periods of *Hypothyris* caterpillars are correlated, so that larval growth rate can be reduced directly (i.e., low temperatures prolong molt period) or indirectly (i.e., low temperatures prolong growth period that implies in a molt period correspondently longer).

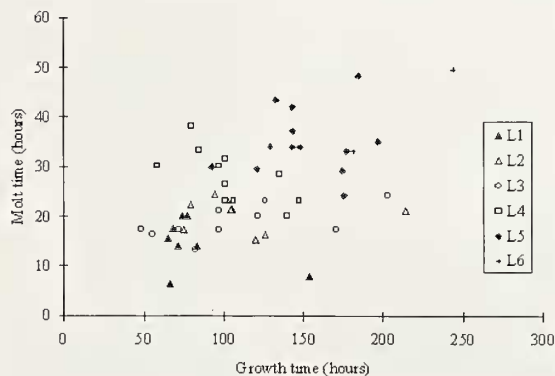
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LITERATURE CITED

- CASEY, T. M. 1993. Effects of temperature on foraging of caterpillars, pp. 5-28. *In*: N. E. Stamp & T. M. Casey (eds.). Caterpillars: ecological and evolutionary constraints on foraging. Chapman, New York.
- COSTA, F. A. P. L. 1991. Sobre a utilização de *Solanum ceriseum* Vell. (Solanaceae) como planta hospedeira por *Hypothyris ninonia daeta* (Bdv., 1836) (Lepidoptera: Nymphalidae: Ithomiinae). MS thesis, Universidade Estadual de Campinas, Campinas.
- COSTA, F. A. P. L. 1996. Demografia foliar: contando folhas no braço-de-preguiça. *Ciência Hoje* 123: 22-24.
- COSTA, F. A. P. L. 1999. New records of larval hostplants for Ithomiinae butterflies (Nymphalidae). *Revista Brasileira de Biologia* 59: 455-459.
- COSTA, F. A. P. L. 2002. Borboletas Ithomiinae (Lepidoptera: Nymphalidae) da Reserva Biológica do Poço D'Anta (Juiz de Fora, MG). *Revista Brasileira de Zoociências* 4: 143-149.
- ESPERK, T., TAMMARU, T. & S. NYLIN. 2007. Intraspecific variability in number of larval instars in insects. *Journal of Economic Entomology* 100: 627-645.
- NIJHOUT, H. F. 1975. A threshold size for metamorphosis in the tobacco hornworm, *Manduca sexta* (L.). *Biological Bulletin* 149: 214-225.
- SOKAL, R. R. & F. J. ROHLF. 1981. *Biometry: the principles and practice of statistics in biological research*, 2 ed. Freeman, San Francisco.

Figure 1. Relationship between growth and molting periods of the same instar (from L1 to L5 plus two extra L6) for 13 *Hypothyris caterpillars*. The linear regression analysis for all these data also produced significant results ($R^2 = 0.2708$; $t = 4.52$; $p < 0.001$). In this figure, seven of the original 57 points are coincident: two full triangles (L1), three empty circles (L3), and two empty quadrats (L4).



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